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DESCRIPTION

SINGLE NUCLEOTIDE POLYMORPHISMS FOR THE DIAGNOSIS AND TREATMENT OF SCHIZOPHRENIA SPECTRUM DISORDERS

FIELD OF THE INVENTION

[0001] This invention relates to the diagnosis and treatment for psychotic and affective disorders, such as schizophrenia. This invention also relates to methods of screening for therapeutics for schizophrenia and other psychotic and affective disorders.

DESCRIPTION OF THE PRIOR ART

[0002] Common human neuropsychiatric disorders including psychotic and affective disorders are multi-factorial or complex in nature. The family of disorders that share clinical and often etiologic (i.e., genetic) characteristics with schizophrenia have been referred to as the "schizophrenia spectrum", which covers schizophrenia, nonschizophrenic, nonaffective psychotic disorders (i.e., schizophreniform disorder, delusional disorder, schizoaffective disorder, atypical psychosis), schizophrenia-like personality disorders and some affective disorders (A. Breier, 1999. Diagnositc classification of the psychoses: historical context and implications for neurobiology. In: Neurobiology of Mental Illness. D. S. Charney, E. J. Nestler, B. S. Bunney (Ed.) Oxford University Press. Chapter 15. pp. 197 – 198). To date, linkage studies have been relatively unsuccessful in identifying genes associated with complex disorders such as bipolar disorder, schizophrenia, depression, anxiety-related traits and autism; few loci have yet been unequivocally identified.

[0003] Schizophrenia is one of the most common and serious psychiatric disorders and is characterised by a profound disruption of cognition and emotion, affecting fundamental human attributes such as language, thought, perception, and sense of self. The disease is accompanied by an array of symptoms which frequently include hallucination and delusion. Studies of the prevalence of schizophrenia in the general population demonstrated that, in general, 1-year prevalence in adults between the ages of 18 to 54 is estimated to be 1.3 percent. Onset generally occurs during young adulthood (mid-20s for men, late-20s for women), although earlier and later onsets do occur. Onset of the disease may be abrupt or gradual, but most people experience some early signs, such as increasing social withdrawal, loss of interest, unusual behavior, or decreases in functioning prior to the beginning of active positive symptoms.

[0004] At this time, a clear consensus as to the etiopathogenesis of schizophrenia has yet to be established. The most popularly held opinion points to the interaction of genetic factors and major

environmental upheaval during development of the brain. Family, twin, and adoption studies support the role of genetic influences in schizophrenia (Kendler KS and Diehl SR, 1993. The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19:261-85; McGuffin P, Owen MJ and Farmer AE, 1995. Genetic basis of schizophrenia. *Lancet* 346: 678-82; Portin P and Alanen YO, 1997. A critical review of genetic studies of schizophrenia. II. Molecular genetic studies. *Acta Psychiatr Scand* 95:73-80). Immediate biological relatives of people with schizophrenia have about a 10-time greater risk than that of the general population. Given prevalence estimates, this translates into a 5 to 10 percent lifetime risk for first-degree relatives (including children and siblings) and suggests a substantial genetic component to schizophrenia (Kety SS, 1987. The significance of genetic factors in the etiology of schizophrenia: results from the national study of adoptees in Denmark. *J Psychiatr Res* 21:423-9; Tsuang MT, 1991. Morbidity risks of schizophrenia and affective disorders among first-degree relatives of patients with schizoaffective disorders. *Br J Psychiatry* 158:165-70). What also bolsters a genetic role are findings that the identical twin of a person with schizophrenia is at greater risk than a sibling or fraternal twin, and that adoptive relatives do not share the increased risk of biological relatives. However, in about 40 percent of identical twins in which one is diagnosed with schizophrenia, the other never meets the diagnostic criteria. The discordance among identical twins clearly indicates that environmental factors likely also play a role. However, despite the evidence for genetic vulnerability to schizophrenia, scientists are only beginning to identify the genes responsible (Kendler KS & Diehl SR, 1993. The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19:261-85; Shifman S, Bronstein M, Sternfeld M *et al.*, 2002. A highly significant association between a COMT haplotyope and schizophrenia. *Am J Hum Genet* 71: 1296-1302). The current consensus is that multiple genes are responsible (Kendler KS, MacLean CJ, O'Neill FA *et al.*, 1996. Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish Study of High-Density Schizophrenia Families. *Am J Psychiatry* 153:1534-40; Kunugi H, Curtis D, Vallada HP *et al.*, 1996. A linkage study of schizophrenia with DNA markers from chromosome 8p21-p22 in 25 multiplex families. *Schizophr Res* 22:61-8; Portin P and Alanen YO, 1997. A critical review of genetic studies of schizophrenia. II. Molecular genetic studies. *Acta Psychiatry Scand* 95:73-80; Straub RE, MacLean CJ, Martin RB, Ma Y, *et al.*, 1998. A schizophrenia locus may be located in region 10p15-p11. *Am J Med Genet* 81:296-301).

[0005] Excessive levels of the neurotransmitter dopamine have long been implicated in schizophrenia, although it is unclear whether the excess is a primary cause of schizophrenia or the result of a more fundamental dysfunction. Recent evidence implicates much greater complexity in the dysregulation of dopamine and other neurotransmitter systems (Grace AA, 1991. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41:1-24; Grace AA, 1992. The depolarization block hypothesis

of neuroleptic action: implications for the etiology and treatment of schizophrenia. *J Neural Transm Suppl* 36:91-131; Olie JP and Bayle FJ, 1997. New chemotherapy approaches to psychoses. *Encephal* Spec 2: 2-9). Some of this research ties schizophrenia to certain variations in dopamine receptors (Nakamura K, 1995. Symptomatologic characteristics and psychopathology of expression of an "attack of altered perception" in schizophrenic patients. *Seishin Shinkeigaku Zasshi* 97:529-50; Serretti A, Macciardi F & Smeraldi E, 1998. Dopamine receptor D2 Ser/Cys311 variant associated with disorganized symptomatology of schizophrenia. *Schizophr Res* 34:207-10), while other research focuses on the serotonin system (Inayama Y, Yoneda H, Sakai T, *et al.*, 1996. Positive association between a DNA sequence variant in the serotonin 2A receptor gene and schizophrenia. *Am J Med Genet* 67:103-5).

[0006] Serotonin is a key neurotransmitter in the central nervous system, and dysregulation of serotonergic pathways has been implicated in the pathogenesis of many complex psychiatric diseases. Polymorphisms of many of the genes involved in serotonin biosynthesis, catabolism, and response have been reported, suggesting that genetic variability may underlie the development of diseases such as schizophrenia, obsessive compulsive disorder, and suicide.

[0007] GABA (γ-aminobutyric acid) is a major neurotransmitter that mediates inhibitory transmission. GABA receptors are divided into two types, $GABA_A$ receptors and $GABA_B$ receptors. $GABA_A$ receptors are the major inhibitory neurotransmitter receptors. They are ligand-gated chloride ion channels that possess binding sites for many important drugs thought to act, in part, through modulation of receptor function (Burt DR and Kamatchi GL, 1991. $GABA_A$ receptor subtypes: from pharmacology to molecular biology. *FASEB* 5: 2916-2923).

[0008] Abnormalities of the GABA system have been implicated in the pathophysiology of schizophrenia (Adel A *et al.*, 1999. Critical review of GABAergic drugs in the treatment of schizophrenia. *J Clin Psychopharmacol* 19: 222-232). Several studies have also shown that in cortical $GABA_A$ receptors, tritiated muscimol binding and levels of α, β_2 and β_3 subunits of the receptor increased. Moreover, the expression of the short isoform of the γ_2 subunit of this receptor may be markedly reduced in the dorsolateral prefrontal cortex (PFC) of schizophrenic subjects (Lewis DA, 2000. GABAergic local circuit neurons & prefrontal cortical dysfunction in schizophrenia. *Brain Research Reviews* 31: 270-276).

[0009] $GABA_A$ receptors contain at least 18 subunits. They are located on the human chromosomes 1, 4, 5, 6, 15 and X. The α, $\beta_{2,3}$ and γ subunit genes of $GABA_A$ receptor are genes that could be related to schizophrenia (Adel A *et al.*, 1999. Critical review of GABAergic drugs in the treatment of schizophrenia. *J Clin Psychopharm* 19: 222-232; Lewis DA, 2000. GABAergic local circuit neurons & prefrontal cortical dysfunction in schizophrenia. *Brain Research Reviews* 31: 270-276; Rapadimitrion G *et al.*, 2001.

Association between $GABA_A$ receptor α_5 subunit gene locus and schizophrenia of a later age of onset. *Neuropsychobiology* 43: 141-144)

[0010] The α_1 subunit is present predominantly in the cerebellum whereas the α_5 subunit is prevalent in the hippocampus, and α_6 subunit is expressed almost exclusively within the cerebellar granule cell. The α_4 subunit constitutes a more minor receptor subtype is expressed primarily in the thalamus and the hippocampus (Mehta AK, Maharaj KT, 1999. An update on $GABA_A$ receptors. *Brain Res Rev* 29: 196-217).

[0011] The β subunit is a key structural and functional component of $GABA_A$ receptors. It was recognized as a potential binding site for GABA (Krishek BJ *et al.*, 1996. Homomeric β_1 γ-aminobutyric $acid_A$ receptor-ion channels: evaluation of pharmacological and physiological properties. *Molecular Pharmacology* 49: 494-504). The importance of β subunit isoforms in governing $GABA_A$ receptor α_1 subunit expression has been suggested (Kumar M *et al.*, 2001. Antisense suppression of $GABA_A$ receptor βs subunit levels in cultured cerebellar granule neurons demonstrates their importance in receptor expression. *J Neurochem* 77: 211-219). The most prevalent β subunit is β_2, which was most often associated with the α_1 subunit (Stephenson FA, 1995. The $GABA_A$ receptors. *Biochem J* 310: 1-9). The β_2 subunit has two isoforms: long form β_{2L} and short form β_{2s}. The $h\beta_{2L}$ cDNA sequence is identical to $h\beta_{2s}$ except for a 114 base pair (38 amino acids) insertion within the large intracellular loop between transmembrane regions III and IV. This 114 base pair insertion sequence was searched for consensus phosphorylation sites and was found to contain a motif for a calmodulin-dependent protein kinase II (CDPK-II) with threonine as the acceptor. It has been proposed that benzodiazepines produce their anticonvulsant effects by inhibiting brain calcium or calmodulin-dependent protein kinase. Therefore, the CDPK-II motif contained in the $h\beta_{2L}$ insert could play a role in determining the pharmacology of $GABA_A$ receptors containing $h\beta_{2s}$ versus $h\beta_{2L}$ (McKinley DD *et al.*, 1995. Cloning, sequence analysis and expression of two forms of mRNA coding for the human β_2 subunit of the $GABA_A$ receptor. *Mol Brain Res* 28: 175-179). Changes in β subunit composition may alter receptor function and pharmacology in vivo. Modulation of receptor function by phosphorylation is also influenced by β subunit composition and contributes to increased inhibition during neuronal excitation (Russek SJ et al., 2000. An initiator element mediates autologous down-regulation of the human type A γ-aminobutyric acid receptor β_1 subunit gene. *Proc Natl Acad Sci* 97: 8600-8605).

[0012] The β_2 subunits are most likely associated with the α_1 subunit (Stephenson FA, 1995. The $GABA_A$ receptors. *Biochem J* 310: 1-9). The whole β_2 sequence (*GABRB2*) could be obtained by re-organising source sequences from the National Center for Biotechnology Information (NCBI) database by BLASTing the unorganised whole gene sequence with long form (NM_021911) and short form (NM_000813) mRNA. Reorganisation of the BLAST result yields the complete sequence and distribution of exons and introns of the β_2 subunit gene. The complete sequence of β_2 subunit gene covers

about 254 kb. The long form transcript variant contains 10 exons totalling 1.5 kb, and the short form transcript variant contains 9 exons totalling 1.4 kb. Exon 9 which contains 113 bases in sequence is deleted from the short form transcript.

[0013] While a number of reports have suggested a correlation between $GABA_A$ receptors and schizophrenia (see above), the exact genetic basis for the correlation remains unclear. It is the object of the present invention to provide further genetic tools and methods to assist the diagnosis and treatment of schizophrenia.

[0014] Single Nucleotide Polymorphisms

[0015] Single nucleotide polymorphisms (SNPs) represent one of the most common forms of genetic variation. These polymorphisms occur when a single nucleotide (A, G, C or T) in the genome is altered. The most common form of SNP is the replacement of cytosine with thymine. SNPs generally tend to be evolutionarily stable from generation to generation and, as such, can be used to study specific genetic abnormalities throughout a population. SNPs often occur in protein coding regions and, as a result, may lead to the expression of a defective or variant form of a protein. Such polymorphisms can therefore serve as effective indicators of genetic disease. However, not all SNPs are found in protein coding regions of the genome. Some SNPs are located in noncoding regions, but these polymorphisms may also lead to altered protein expression. Specifically, SNP sites in noncoding regions may, for example, lead to differential and defective splicing. In diseases such as schizophrenia, where a large number of genes may influence the onset of the disease, SNPs can be used as diagnostic tools for identifying individuals with a predisposition for manifesting the disease, genotyping the patients suffering from the disease in terms of the genetic causes underlying the condition, and facilitating drug development based on the insight revealed regarding the role of target proteins in the pathogenesis process.

[0016] When SNPs are identified and genotyped in patients or in families, data must be analyzed to determine whether the association between an SNP and a disease is significant. Just as linkage is a relationship between loci, association is a relationship between alleles. Thus, an alternative to linkage mapping in families is to search for statistical associations between one allele and the disease. The idea behind this approach is that if one allele m1 can directly cause susceptibility to the disease, generally, possession of m1 is generally not necessary or sufficient for someone to develop the disease, but its frequency should increase in the proband population compared to unaffected controls. The same should also be true for all alleles in linkage disequilibrium (LD) with allele m1. Thus, SNP-based association studies can be performed in two ways: direct testing of a SNP with functional consequence for association, or using a SNP as a marker for LD. Therefore, a common core haplotype involving several SNPs should be more frequent in probands compared to controls.

[0017] The present invention seeks to provide a set of SNPs and their haplotypes for diagnoses of psychotic and affective disorders such as schizophrenia and identifying appropriate target proteins for drug development.

SUMMARY OF THE INVENTION

[0018] In one embodiment, the present invention provides an isolated polynucleotide having a nucleotide sequence comprising corresponding to SEQ ID NO: 1, the polynucleotide comprising a portion of the human *GABRB2* gene including a polymorphic site at position 1584 of intron 7.

[0019] In another embodiment, the invention provides a PCR primer set for amplifying regions of a polynucleotide corresponding to SEQ ID NO: 1, the primer set comprising a first primer having a sequence corresponding or complementary to a sequence corresponding to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 or SEQ ID NO: 12, and a second primer having a sequence corresponding or complementary to a sequence corresponding to SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13.

[0020] In yet another embodiment, the invention provides a method of predicting whether an individual is more or less likely to suffer from schizophrenia comprising:

a) determining the nucleotide sequence from a nucleic acid sample from the individual; and

b) identifying DNA sequences corresponding to one or more SNPs selected from the group consisting of: a novel SNP I7G1584T, and five known SNPs reported in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), namely rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of the SNPs.

[0021] In yet another embodiment, the present invention provides a method of genotyping the schizophrenia-spectrum disorder of a patient in order to provide pharmacogenetic guidance for drug therapy for the patient, comprising:

[0022] a) determining the nucleotide sequence from a nucleic acid sample from a schizophrenic patient; and

[0023] b) identifying DNA sequences corresponding to one or more SNPs selected from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of the the SNPs.

[0024] In another embodiment, the invention provides a method of drug screening for identifying drugs for treating schizophrenic disorders, the method comprising:

a) transfecting a vector into a eukaryotic expression system, the vector containing a DNA sequence

corresponding to at least a portion of the human *GABRB2* gene, the portion of the *GABRB2* gene including one or more SNPs chosen from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269;

b) expressing the vector in a cellular expression system;

c) adding a drug to be screened into the cellular system;

d) analyzing the expression of the *GABRB2* gene encoding $GABA_A$ receptor β_2 subunit and also analysing the expression and resultant activity of the $GABA_A$ receptor in the cellular system;

e) determining the effect of the drugs on the expression of the $GABA_A$ β_2 subunit and the $GABA_A$ receptor, and the activity of the expressed $GABA_A$ receptor in the cellular system; and,

f) identifying those drugs that alter the levels of the expression of *GABRB2* gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

[0026] Figure 1a is a nucleotide sequence of Exon 7, Exon 8 and the Intron 7 between such exons of the human *GABRB2* gene comprising the three SNPs I7G1584T, rs1816071 and rs1816072.

[0027] Figure 1b is a nucleotide sequence of Exon 7, Exon 8 and the Intron 7 between such exons of the human *GABRB2* gene comprising the two SNPs rs194072 and rs252944.

[0028] Figure 2 is a nucleotide sequence of Exon 8, Exon 9 and the Intron 8 between such exons of the human *GABRB2* gene comprising the SNP rs187269.

[0029] Figure 3 shows the locations of the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene.

[0030] Figure 4 shows data obtained from the sequencing gel of the flanking regions around the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0031] The below terms are used throughout the present application and will be assumed to have the following definitions:

[0032] The term "allele" is used herein to refer to variants of a nucleotide sequence.

[0033] The term "antisense" is used herein, to refer to a complementary strand of a coding sequence of DNA or RNA. Such coding sequence may contain, for example, a SNP of interest. Copies of antisense polynucleotides can be introduced into a cell or organism to inhibit expression of a corresponding gene or to

alter the splicing of mRNA in specific regions. Antisense polynucleotides are entirely or substantially complementary to a target polynucleotide and have the ability to specifically hybridize to such target polynucleotide.

[0034] The terms "complementary" and "complement", as used herein, refer to polynucleotide sequences which are capable of base pairing with another polynucleotide throughout the complementary region.

[0035] The term "haplotype" refers to a series of known DNA sequences linked on a chromosome. The known DNA sequences can be in the form of SNPs or other markers. A haplotype may refer to a combination of polymorphisms found in an individual that may be associated with a phenotype.

[0036] The term "genotype" refers to the genetic constitution of an organism. More specifically, the term refers to the identity of alleles present in an individual. "Genotyping" of an individual or a DNA sample refers to identifying the nature, in terms of nucleotide bases, of a specific allele possessed by an individual at a known polymorphic site.

[0037] The term "polymorphism", as used herein, refers to the coexistence of more than one form of gene or portion thereof in a population. "Polymorphic" refers to the condition in which two or more variants of a specific genomic sequence can be found in a population. A "polymorphic site" refers to a locus at which variations in nucleotide sequence occur.

[0038] The terms "oligonucleotide" and "polynucleotide" as used in the present application refer to RNA, DNA or hybrid RNA/DNA sequences being of greater than one nucleotide in length. Such sequences may exist in either single or double-stranded form.

[0039] The term "PCR", as used herein, refers to the polymerase chain reaction. PCR is a method of amplifying a DNA base sequence using a heat stable polymerase and a pair of primers, one primer complementary to the (+)-strand at one end of the sequence to be amplified and the other primer complementary to the (-) strand at the other end of the sequence to be amplified. Newly synthesized DNA strands can subsequently serve as templates for the same primer sequences and successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR can be used to detect the existence of a defined sequence in a DNA sample.

[0040] The term "primer", as used herein, refers to a short single-stranded oligonucleotide capable of hybridizing to a complementary sequence in a DNA sample. The primer serves as an initiation point for template dependent DNA synthesis. Deoxyribonucleotides can be added to a primer by a DNA polymerase. A "primer pair" or "primer set" refers to a set of primers including a 5'upstream primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3', downstream primer that hybridizes with the complement of the 3' end of the DNA sequence to be amplified.

[0041] The term "exon" refers to the protein coding DNA sequence of a gene.

[0042] The term "intron" or "intronic region" refers to DNA base sequences interrupting the protein-coding sequences of a gene. These sequences are transcribed into RNA but are cut out of the RNA before it is translated into protein.

[0043] The present invention relates, generally, to single nucleotide polymorphisms (SNPs) associated with the etiopathogenesis of schizophrenia. As found by the present inventors, individuals possessing certain SNPs, or their complementary regions, have been found to have an increased susceptibility to a schizophrenia spectrum disorder. These disorders have been linked to mutations of the *GABRB2* gene. As indicated above, the *GABRB2* gene encodes the β_2 subunit of human gamma-aminobutyric acid type A receptor.

[0044] In one aspect, the present invention provides an association of certain SNP sites with schizophrenia disorders. These SNPs are as follows: rs1816071, rs1816072, rs194072, rs252944, and rs187269. These SNPs are present in the introns preceding and following Exon 8 of *GABRB2*, and the haplotype or haplotypes containing one or more of these six SNPs. The above listed SNPs are known in the art but have not been previously associated with schizophrenia disorders.

[0045] According to another embodiment, the present invention provides a further, previously unknown SNP, also associated with schizophrenia disorders. This SNP, referred to herein as SNP I7G1584T, is found in intron 7 of the *GABRB2* gene. This intron is the region between Exons 7 and 8 of the aforementioned gene. The methodology used to identify this SNP is provided below.

[0046] Results of linkage disequilibrium or association analysis of the above mentioned six SNPs are shown in Tables 1 and 2(provided herein below), which present data concerning the correlation between each allele and genotype frequency with schizophrenia. In Tables 1 and 2, "SCH" represents schizophrenic patients and "CON" represents normal controls. Statistical testing of the data shows that the six SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 in the β_2 subunit gene are significantly associated at the allelic level with schizophrenia, with $p < 0.05$; and that the five SNPs I7G1584T, rs1816071, rs194072, rs252944 and rs187269 are significantly associated at the genotypic level with schizophrenia, with $p < 0.05$. Therefore, these various SNPs may serve as potential genetic factors linked to the etiopathogenesis of schizophrenia.

[0047] Accordingly, in one aspect, the present invention provides a method of detecting the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944, and rs187269, and their associated haplotypes, for the diagnosis of susceptibility to schizophrenia, the genotyping of cases of schizophrenia, and to render $GABA_A$ receptors containing a β_2 subunit as potentially useful target protein systems for the screening and development of drugs to treat the disease.

[0048] Figure 1a depicts a nucleotide sequence containing Exon 7, Exon 8 and the Intron 7 between such exons in the human *GABRB2* gene. In Figure 1a, the SNP I7G1584T is identified by a rectangular box with solid lines. The SNP rs1816071 is identified by a rectangular box with dashed lines, and the SNP rs1816072 is identified by a rectangular box with a double solid line. Exon 7 is underlined with a double dashed line. Exon 8 is underlined with a single dashed line. The PCR-amplified region containing I7G1584T, rs1816071 and rs1816072 is underlined with a solid line.

[0049] In Figure 1b, the SNP rs194072 is identified by a rectangular box with solid lines. The SNP rs252944 is identified by a rectangular box with dashed lines. Exon 7 is underlined with a double dashed line. Exon 8 is underlined with a single dashed line. The PCR-amplified region containing rs194072 is underlined with a solid line. The PCR-amplified region containing rs252944 is shown in bold type.

[0050] Referring now to Figure 2, a nucleotide sequence of Exon 8, Exon 9 and the Intron 8 between such exons of the human *GABRB2* gene is presented. The SNP rs187269 is identified by a rectangular box with a solid line. Exon 8 is shown in bold type and Exon 9 is underlined with a dashed line. The PCR-amplified region is underlined with a solid line.

[0051] The above-mentioned figures present the SNPs which have been associated with the schizophrenia.

[0052] It is another aspect of the present invention to provide a method of screening and diagnosing the occurrence and genotype of schizophrenia by analyzing the sequences of the GABA receptor β_2 gene, namely *GABRB2*, in order to identify the allele and/or genotype and/or haplotype of the subject at SNP I7G1584T, rs1816071, rs1816072, rs194072 or rs252944 or rs187269 or a combination including any number of these six SNPs. The method includes obtaining human DNA in sufficient quantity for sequence analysis, and identifying the nature of the base at one or more of these SNP positions in the human *GABRB2* gene. The method used may involve common techniques such as PCR, under the same conditions and using the same primers as described below in the examples provided for detection of nucleotide sequences associated with schizophrenia, followed by DNA sequence analysis. Alternate primers upstream and downstream of these six SNP positions may also be used and may be determined by one skilled in the art without undue experimentation. Any other methods useful for SNP genotype and haplotype analysis may be applied to identify the variants at the six SNP sites and other markers in the same haplotype as, or in linkage disequilibrium with, the six SNPs.

[0053] The examples contained in the present application include methods for screening and/or diagnosing schizophrenic disorders. In summary, the invention provides that if the base T is found at I7G1584T, the subject may be at higher risk for developing schizophrenia than if G is found at this position.

If the base G is found at rs1816071, the subject may be at higher risk for developing schizophrenia than if A is found at this position. If the base C is found at rs1816072 and/ or rs194072 and/ or rs187269, the subject may be at higher risk for developing schizophrenia than if T is found at this position. Likewise, if the base C is found at position rs252944, the subject may be at a higher risk for developing schizophrenia than if G is found at this position.

[0054] Referring to Figure 3, a schematic diagram presenting the distribution of exons in the genome nucleotide sequence of the human *GABRB2* gene is provided. The figure depicts the locations of the SNP sites I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 in *GABRB2*, which can be used in an embodiment of the present invention for determining if an individual has a predisposition for schizophrenia.

[0055] The above mentioned polymorphisms can be used in diagnostic tests in order to identify individuals who have a predisposition for developing schizophrenia. Such diagnostic tests may utilize a number of technologies known in the art including, use of DNA chip methods (such as GenFlex™ Tag, Affymetrix) and DNA immobilization on beads (such as SIGNET™ Y-SNP Identification System, Marligen Bioscience, Inc.), ABI Prism® SNPaPshot™ Multiplex System (Applied Biosystems Inc.) and Pyrosequencing™ (Pyrosequencing AB). Diagnostic tests can also be conducted using allele specific primers, allele specific probes, directing sequencing of the intron of interest, and denaturing gradient gel electrophoresis. The present invention therefore provides a method of diagnosing an individual with schizophrenia, or an individual with a predisposition to schizophrenia, by determining the presence or absence of a *GABRB2* haplotype. Such haplotype would include the SNP sites I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269.

[0056] In yet another aspect of the present invention, synthetic or recombinant DNA molecules are provided for diagnostic purpose. Such molecules comprise a sequence encompassing one or two or three of the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, in one or both their allelic forms, and their flanking regions, and any haplotype or haplotypes associated with one or more of these six SNPs, as well as any genetic locus or loci in linkage disequilibrium with the six SNPs, with respect to one or both DNA strands of the *GABRB2* gene.

[0057] In an additional embodiment of the present invention, a method is provided for screening therapeutic drugs for treatment of schizophrenia, based on the correlation between the novel SNP I7G1584T, the SNPs rs1816071, rs1816072, rs194072, rs252944, and rs187269, and the disease. Because these six SNPs are located in the intron regions of *GABRB2*, they may modulate the synthesis mRNA splicing and the activity of $GABA_A$ receptors containing the β_2 subunit. To detect such

modulation, a *GABRB2* gene containing different combinations of the different allelic forms of these six SNPs may be cloned, and expressed along with other $GABA_A$ subunit genes, by means of appropriate expression vectors in cell lines such as P12 (ATCC, The Global Bioresource Center™), and using commonly used transfection kits such as the CellPhect Transfection Kit (Amersham Pharmacia Biotech,Inc.,USA), and ProFection® Mammalian Transfection Systems (Promega Coporation). The cell line may or may not be of neuronal origin. Transfection efficiency can be enhanced by using a number of reagents known in the art including, for example, calcium phosphate (Life Technologies Inc.).

[0058] In the event that one or more of the SNP's I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187289, in their schizophrenia-associated allelic form, should alter the expressed activity of $GABA_A$ receptor through a modification of the short form/long form ratio, some structural feature, or the amount of the expressed β_2 subunit, the above mentioned cellular system also may be employed to screen and search for compounds that can restore the normal level of expressed activity of $GABA_A$ receptor. Such compounds may be potentially useful candidate drugs for the treatment of schizophrenia.

[0059] The invention further provides kits for detection of SNP sites associated with schizophrenia.

[0060] To detect the SNPs of interest by DNA sequencing or other genotyping methods, a PCR is performed to amplify the DNA segment of a test subject containing one or more of the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, and the PCR products are subjected to DNA sequencing or other genotyping methods such as single-base extension or microarray-based sequence-specific oligonucleotide probe hybridization, to determine the allelic form or forms of the polymorphism(s). The results may be analysed by computer software packages such as POLYPHRED™ and CONSED™ for DNA sequencing data, or other suitable software. A kit that may serve such detection may contain, for example, one or more of the following components including: PCR primers, allele specific probes, DNA polymerase, PCR reaction buffer, magnesium chloride and four deoxynucleotides for use in a PCR reaction or extension of probe sequences. In addition, if a sequencing reaction is conducted a kit may also include, sequencing primers, DNA polymerase, four labelled dideoxynucleotides, and four un-labelled deoxynucleotides.

[0061] In cases where genotyping methods do not require PCR amplification of DNA are employed, the DNA of a test subject can be directly used in experimental characterization of the six SNPs, and the haplotype or haplotypes associated with the six SNPs.

[0062] The present invention also provides a method of drug screening comprising the steps of:

[0063] (a) Transfecting a vector into a eukaryotic expression system, the vector containing the DNA sequence corresponding to part or all of the human *GABRB2* gene, by itself or along with other genes encoding the proteins of the human $GABA_A$ receptor;

[0064] (b) Expressing the vector in the cellular expression system;

[0065] (c) Adding a drug to be screened into the cellular system

[0066] (d) Analyzing the gene expression of the *GABRB2* gene encoding the $GABA_A$ receptor β_2 subunit, the *GABRB2* gene being in all possible allelic combinations relating to the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, and also analysing the expression and resultant activity of the $GABA_A$ receptor in the cellular system.

[0067] (e) Determining the effect of chemical compounds or preparations on the cellular system with respect to the expression of $GABA_A$ β_2 subunit and the $GABA_A$ receptor, and the activity of the expressed $GABA_A$ receptor in the cellular system.

[0068] The chemical compounds or preparations so identified are potentially useful candidate drugs for the treatment of schizophrenia.

[0069] While the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269 may be used singly or jointly for diagnostic or drug development purposes, one or more genetic markers belonging to the same haplotype as, or in linkage disequilibrium with, one or more of these six SNPs, may also be employed for these purposes.

[0070] Furthermore, while the PCR and DNA sequencing methods described below in Example 1, and similar methods such as DNA sequencing by means of pyrosequencing (Pyrosequencing AB), can reveal the genotype of a test DNA sample in terms of the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, alternative methods can also be employed for this purpose. Such alternate methods include the use of DNA chip methods (such as GenFlex™ Tag, Affymetrix) and DNA immobilization on beads (such as SIGNET™ Y-SNP Identification System, Marligen Bioscience, Inc.), instead of DNA sequencing, and methods using single DNA molecule sequencing instead of PCR (such as ABI Prism® SNPaPshot™ Multiplex System, Applied Biosystems Inc.).

[0071] The examples presented below are provided to illustrate the present invention and are not meant to limit the scope of the invention as will be apparent to persons skilled in the art.

[0072] **EXAMPLE 1: Identification of the Novel SNP site of the Invention:**

[0073] The novel SNP I7G1584T of the invention was identified in the method as described below.

[0074] For discovery of SNPs within the region of interest, specific primers were designed in an adjacent-primer-overlapping-200-base-pair manner by using the online primer designation program, Primer3™ (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The specificity of each suggested primer to the human genome was checked through BLAST™ (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/BLAST). Only both forward and reverse primers with less than 5 hits to human genome were considered as specific primers and employed.

[0075] PCR was preformed in a final volume of 20 µl containing: 10 ng sample DNA from either controls or patients; 0.075 µmole/L of each primer; 500 µmol/L of each dNTP; 2.5 mmol/L $MgCl_2$; and 1U *Taq*™ polymerase (Amersham Pharmacia Biotech, Inc, USA). Gradient PCR was engaged initially to determine the optimum annealing temperature for each pair of primers. Examples of primer sequences and their optimum annealing temperatures are listed in Table 3. PCR amplification consisted of denaturation at 94°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at optimum annealing temperature for each pair of primers to DNA template, 1.5 min at 72°C and a final extension step at 72°C for 5 min. PCR products were then resolved using 1.5% agarose gel electrophoresis and staining with ethidium bromide to confirm desired and specific products generated. PCR products were purified by MultiScreen™ PCR_{96} Purification kit (Millipore Corporation, Bedford, USA).

[0076] Discovery of SNPs within the region of interest were preformed by auto-sequencing of PCR fragments on both strands. The sequencing reactions contained: 2 µl BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems); 5µl purified PCR products; and 1 mmol/L of primer. Sequencing cycling conditions consisted of 1 min denaturation at 96°C followed by 34 cycles of 96°C for 30sec, 50°C for 30sec and 60°C for 3min. Sequencing products were purified by AutoSeq96™ Plates containing DNA Grade Sephadex™ G-50 (Amersham Pharmacia Biotech, Inc, USA). Purified sequencing products were then denatured at 95°C for 5 min with addition of 5µl Hi-Deionized Formamide. Denatured sequencing products were run on the ABI Prism model 3100 DNA sequencer according to ABI protocols.

[0077] Sequence data were aligned and analyzed for SNPs by POLYPHRED™ software (Nickerson DA, Tobe VO & Talylor SL, 1997 Nucleic Acids Res. 15; 25: 2745-51) and CONSED™ software (Gordon D, Abajian C and Green P, 1998 Genome Res. 8: 195-202).

EXAMPLE 2: Detection of Nucleotide Sequences Associated with Schizophrenia

[0078] This example describes the protocol utilized to find and test for the presence of one or more the above-mentioned SNPs. In this example, PCR-amplified products are synthesized to encompass the target regions of human $GABA_A$ receptor β_2 subunit of the *GABRB2* gene. A pair of primers, specific to each of these regions, and which can be used for amplification, are shown in Table 3.

Table 3 The Primers of β_2 Subunit Genes for Both PCR and DNA Sequencing

Cluster ID of dbSNP	Forward primer	Reverse primer	Optimum annealing temperature for PCR

I7G1584T	atggaggaaaggtccatatctag t (SEQ ID NO:12)	cctctaagctgtaatcggaaggt a (SEQ ID NO:13)	62°C
rs1816071	atggaggaaaggtccatatctag t (SEQ ID NO:10)	cctctaagctgtaatcggaaggt a (SEQ ID NO:11)	62°C
rs1816072	atggaggaaaggtccatatctag t (SEQ ID NO:8)	cctctaagctgtaatcggaaggt a (SEQ ID NO:9)	62°C
rs194072	taccttccgattacagcttagag g (SEQ ID NO:2)	tggagaggactctaggtcaactt t (SEQ ID NO:3)	64°C
rs252944	cacaatgatttttccgagaccat (SEQ ID NO:4)	ttgtaaagctattgtccagcaag t (SEQ ID NO:5)	62°C
rs187269	agcacttgctgcactaacagaat a (SEQ ID NO:6)	agacaatgcctaatgtcctctgg (SEQ ID NO:7)	62°C

[0079] **PCR Amplification of SNP Containing Regions and Sequencing of Amplified Regions**

[0080] The following reagents were used for amplification reactions for amplifying the regions of interest: 2 µl of PCR buffer (Amersham Pharmacia Biotech, Inc, USA); 2.5 mM $MgCl_2$; 500 µM dNTPs; 0.075 µM of each primer; 10 ng DNA; and 1 U Taq™ DNA polymerase (Amersham Pharmacia Biotech, Inc., USA); in a total volume of 20 µl.

[0081] PCR amplification was performed in the PTC-200™ Peltier thermal-cycler (MJ Research, Inc.) and RoboCycler™ (Stratagene, USA) using 96-well microplates under the following thermal cycle conditions: 5 minutes at 94 °C for denaturation; and 40 cycles for optimal DNA amplification that entailed denaturation at 94 °C for 30 seconds, annealing at 60 °C – 62 °C for 30 seconds, and polymerization at 72 °C for 1.5 minutes.

[0082] The PCR products were purified using a MultiScreen®-PCR kit, (Millipore Corporation, Bedford, USA), and were directly sequenced using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) under the following conditions: 96 °C for 1 minute followed by 34 cycles of 96 °C for 30 seconds, 50 °C for 15 seconds, and 60 °C for 3 minutes.

[0083] Sequencing products were purified by AutoSeq96™ Plates containing DNA Grade Sephadex™ G-50 (Amersham Pharmacia Biotech, Inc, USA). Purified sequencing products were then denatured at 95°C for 5 min with addition of 5µl Hi-Deionized Formamide. Denatured sequencing products were run on the ABI Prism model 3100 DNA sequencer according to ABI protocols. Sequence data were aligned and analyzed for SNPs by POLYPHRED™ software (Nickerson DA, Tobe VO & Talylor SL, 1997 Nucleic Acids Res. 15; 25: 2745-51) and CONSED™ software (Gordon D, Abajian C

and Green P, 1998 Genome Res. 8: 195-202). Each PCR product was sequenced from both ends using the same primers as those utilized for the DNA amplification described above.

[0084] The DNA sequencing results using this procedure are shown in Table 1. This table shows the nature of the SNP bases in samples from control (CON) and schizophrenic (SCH) subjects in term of the allelic form of the six SNPs examined.

[0085] Although one method of detecting the six polymorphic sites associated with schizophrenia is provided above, it will be understood by persons skilled in the art that other detection methods may also be utilized. For example, detection can also be accomplished, inter alia, through allele specific probes designed to identify the presence or absence of an individual SNP site, allele specific primer extension reactions, and single base primer extension reactions.

[0086] **EXAMPLE 3: Genotyping of I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 polymorphisms**

[0087] For the novel SNP I7G1584T, and the five SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269, the DNA sequencing results in Table 1 from healthy Controls, and Schizophrenics satisfying DSM IV criteria (American Psychiatric Association, 1994) were analysed by genotyping in order to determine whether the aforementioned SNPs were associated with an increased risk of schizophrenia. Identification of SNPs was conducted using the primers and sequencing method as described in Example 2.

[0088] **Table 1: Forms of SNPs in the *GABRB2* Gene**

Cluster ID of dbSNP	Allele			Genotype		
		CON	SCH		CON	SCH
I7G1584T	G	163	185	G/G	72	72
				G/T	19	41
	T	21	55	T/T	1	7
	Total	184	240	Total	92	120
rs1816071	A	137	142	A/A	56	51
				A/G	25	40
	G	37	82	G/G	6	21
	Total	174	224	Total	87	112

rs1816072	T	131	125	T/T	44	37
				T/C	43	51
	C	71	105	C/C	14	27
	Total	202	230	Total	101	115
rs194072	T	173	184	T/T	77	73
				T/C	19	38
	C	23	56	C/C	2	9
	Total	196	240	Total	98	120
rs252944	G	240	148	G/G	104	55
				G/C	32	38
	C	36	52	C/C	2	7
	Total	276	200	Total	138	100
rs187269	T	284	188	T/T	117	70
				T/C	50	48
	C	58	74	C/C	4	13
	Total	342	262	Total	171	131

[0089] As shown in Table 2, the Chi-square test for comparing the genotypes of Controls (CON) and Schizophrenics (SCH) shows a very significant increase of homozygote T/T carriers of novel SNP I7G1584T, homozygote G/G carriers of rs1816071 and homozygote C/C carriers of rs1816072, rs194072, rs252944 and rs187269 among schizophrenics. The comparison of allele frequencies also indicates a highly significant increase in T of novel SNP I7G1584T, G of rs1816071 and C of rs1816072, rs194072, rs252994 and rs187269 in Schizophrenics in comparison to Controls.

[0090] Table 2 shows the frequency distribution of genotypes and alleles for the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269 polymorphisms. Table 2 shows the *p* values from the Chi-square test, odds ratios (OR) and 95% confidence interval (95% CI) in comparing the following samples: a) 120 Schizophrenics (SCH) and 92 Controls (CON) for novel SNP I7G1584T; b) 112 Schizophrenics and 87 Controls for rs1816072; c) 115 Schizophrenics and 101 Controls for rs1816072; d) 120 Schizophrenics and 98 Controls for rs194072; e) 100 Schizophrenics and 138 Controls for rs252944; and f) 131 Schizophrenics

and 171 Controls for rs187269. All the Controls and Schizophrenics were *Han* Chinese. The statistic software Prism3™ version 3.02 was used for allele and genotype significance testing.

[0091] Table 2: Association of SNPs in the *GABRB2* Gene with Schizophrenia.

	Allele					
Cluster ID of dbSNP	I7G1584T	rs1816071	rs1816072	rs194072	rs252944	rs187269
p Value	0.0022	0.0009	0.0266	0.0018	0.0003	0.0009
OR	2.308	2.138	1.55	2.289	2.342	1.927
95% CI	1.338-3.981	1.358-3.366	1.051-2.285	1.350 - 3.882	1.460-3.755	1.305-2.847
	Genotype					
Cluster ID of dbSNP	I7G1584T	rs1816071	rs1816072	rs194072	rs252944	rs187269
p Value	0.011	0.0109	0.1044	0.0125	0.0018	0.0031
OR	--	--	--	--	--	--
95% CI	--	--	--	--	--	--

[0092] Table 4 below shows the estimated frequencies for certain haplotypes of the six SNPs and the significance of their association with schizophrenia (SCH) in comparison to unaffected controls (CON). Maximum likelihood haplotype frequencies between possible SNP haplotypes were estimated by the Expectation-Maximization algorithm (Slatkin M, Excoffier L, 1996). Testing for linkage disequilibrium in genotypic data using the expectation-maximization algorithm. Heredity 76:377-383) using the program SNPHAP™ (http://www.hgmp.mrc.ac.uk/Menu/Help/snphap) under Hardy-Weinberg assumption of gamete independence. Association analyses were performed by comparing the haplotype frequencies of Controls and Schizophrenics through the Chi-square test. Association of schizophrenia and estimated two-loci and six-loci haplotype were accessed by CLUMP™ (http://www.hgmp.mrc.ac.uk/Registered/Help/clump/) (Sham PC and Curtis D, 1995. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 59: 97-105) to yield the Chi-square value and its corresponding *p* value.

[0093] The novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072 and rs252944 SNP are located between Exons 7 and 8, and the rs187269 SNP is located betweens Exon 8 and 9. Exon 9 is present in the long form variant of the β_2 subunit of $GABA_A$ receptor mRNA but absent from the short form variant. These six SNPs are thus located close to a region that may play a role in modulating the properties and/or quantity of β_2 incorporated into $GABA_A$ receptors, including but not limited to the alternative splicing of the *GABRB2* mRNA to yield the short form versus the long form of β_2.

Table 4: Haplotype Frequencies and Significance of Association with Schizophrenia

Haplotype	Frequency %		χ^2,df*	*P* (OR; 95%CI)
	CON	SCH		
I7G1584T-rs1816071			16.95, 3	0.00074
G-A	76.81	59.85		
G-G	11.78	18.02		
T-A	0.00	3.75		
T-G	11.41	18.38		
T-G vs. G-A + G-G + T-A			3.70, 1	0.054 (1.725; 0.9855-3.019)
I7G1584T-rs187269			12.89, 3	0.0049
G-T	81.70	67.38		
G-C	6.9	11.36		
C-T	1.45	3.10		
C-C	9.95	18.16		
C-C vs. GT + G-C + C-T			6.738, 1	0.0094 (2.04; 1.182-3.523)
rs1816071-rs1816072			17.44, 3	0.000583
A-T	66.71	48.02		
A-C	12.36	15.76		
G-T	1.31	6.16		
G-C	19.62	30.07		
G-C vs. A-T + A-C + G-T			5.44, 1	0.0197 (1.733; 1.089-2.758)
rs1816071-rs187269			22.90	0.000043
A-T	75.0	55.13		
A-C	2.51	9.55		
G-T	8.98	13.73		
G-C	13.51	21.59		
G-C vs. A-T + A-C + G-T			5.45	0.0197 (1.774, 1.092-2.883)
rs1816072-rs194072			12.73, 3	0.0053
T-T	63.55	52.89		
T-C	0.78	1.72		
C-T	23.95	21.97		
C-C	11.71	23.42		
C-C vs. T-T + T-C + C-T			11.45, 1	0.00072 (2.284; 1.404-3.716)
rs194072-rs252944			8.39, 3	0.03868
T-G	85.23	73.85		
T-C	0.38	1.24		
C-G	0.46	1.03		
C-C	13.92	23.89		

C-C vs. T-G + T-C + C-G			6.77, 1	0.009281 (1.906; 1.167-3.112)
rs252944-rs187269			26.24, 3	0.000009
G-T	80.10	69.45		
G-C	6.79	7.76		
C-T	4.18	0.72		
C-C	8.93	22.06		
C-C vs. G-T + G-C + C-T			20.87, 1	0.000005 (2.960; 1.829-4.808)
rs194072-rs187269			26.83, 3	0.000007
T-T	78.62	68.01		
T-C	5.96	8.36		
C-T	5.30	0.64		
C-C	10.12	22.98		
C-C vs. T-T + T-C + C-T			17.89, 1	0.000024 (2.624; 1.660-4.148)
rs194072-rs252944-rs187269			30.72, 7	0.000072
T-T-T	80.39	65.17		
T-C-C	5.54	7.55		
T-C-T	0.00	0.00		
T-C-C	0.00	1.09		
C-T-T	0.00	0.00		
C-T-C	0.45	1.08		
C-C-T	4.89	1.19		
C-C-C	8.36	23.89		
C-C-C vs others			17.21, 1	0.000034 (2.857; 1.713-4.766)
I7G1584T-rs194072-rs252944-rs 187269			29.29, 15	0.015
G-T-T-T	81.97	65.69		
G-T-T-C	09.13	7.09		
G-T-C-T	0.00	0.00		
G-T-C-C	0.00	1.23		
G-C-T-T	0.00	0.81		
G-C-T-C	0.00	1.50		
G-C-C-T	1.85	0.02		
G-C-C-C	0.59	1.25		
T-T-T-T	0.00	1.33		
T-T-T-C	0.00	0.00		
T-T-C-T	0.00	0.00		
T-T-C-C	0.00	0.00		
T-C-T-T	0.00	0.00		
T-C-T-C	0.00	0.00		
T-C-C-T	2.18	1.40		
T-C-C-C	4.29	19.68		
T-C-C-C vs others			12.79, 1	0.0003 (4.860; 1.888-12.51)

Note: df= degree of freedom

[0094] **EXAMPLE 4: Method for Screening Enhanced Susceptibility to Schizophrenia and for Genotyping Schizophrenics**

[0095] Example 2 describes a method for determining the nature of the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269 in Control subjects and Schizophrenic patients. Example 3 shows that a base T in SNP I7G1584T, a base G in rs1816071 and a base C in rs1816072, rs194072, rs252944 and rs187269 signifies an increased risk of schizophrenia. Furthermore, these six SNPs provide a basis for screening for enhanced susceptibility for schizophrenia, and genotyping schizophrenia patients with the aim of applying pharmacogenetics to determine the optimal therapeutic to be employed for their treatment.

[0096] The same procedures employed in Example 2 also can be performed on test subjects with unknown susceptibility to schizophrenia or schizophrenic patients with unknown genotype. In either case, the procedures employed will reveal the genotype of the test DNA sample with respect to the bases at the SNP positions the novel SNP I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269. The resultant genotype in turn will indicate whether the test subject carries enhanced genetic susceptibility to schizophrenia. In the case of a patient known to be a schizophrenic, the genotype will provide a basis for diagnostic classification and for pharmacogenetic profiling towards achieving optimizing therapy.

[0097] To perform genotyping using the PCR and sequencing procedures as described in Example 2, the following sequences, or sequences similar to them, will be required.

[0098] **Description of Sequence Listings**

[0099] SEQ ID NO:1: The native DNA sequence of Exon 7, Intron 7, Exon 8, Intron 8 and Exon 9 of the human *GABRB2* gene, with variation noted at positions 1584, 1803, 2103, 3106 and 3424 of intron 7, and position 1265 of Intron 8

[00100] SEQ ID NO:2 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 3106 of Intron 7, corresponding to rs194072, of the human *GABRB2* gene.

[00101] SEQ ID NO:3 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 3106 of Intron 7, corresponding to rs194072, of the human *GABRB2* gene.

[00102] SEQ ID NO:4 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 3424 of Intron 7, corresponding to rs252944, of the human *GABRB2* gene.

[00103] SEQ ID NO:5 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 3424 of Intron 7, corresponding to rs252944, of the human *GABRB2* gene.

[00104] SEQ ID NO:6 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 1265 of Intron 8, corresponding to rs187269, of the human *GABRB2* gene.

[00105] SEQ ID NO:7 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 1265 of Intron 8, corresponding to rs187269, of the human *GABRB2* gene.

[00106] SEQ ID NO:8 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 2103 of Intron 7, corresponding to rs1816072, of the human *GABRB2* gene.

[00107] SEQ ID NO:9 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 2103 of Intron 7, corresponding to rs1816072, of the human *GABRB2* gene.

[00108] SEQ ID NO:10 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 1803 of Intron 7, corresponding to rs1816071, of the human *GABRB2* gene.

[00109] SEQ ID NO:11 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 1803 of Intron 7, corresponding to rs1816071, of the human *GABRB2* gene.

[00110] SEQ ID NO:12 is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 1584 of Intron 7, corresponding to I7G1584T, of the human *GABRB2* gene.

[00111] SEQ ID NO:13 is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 1584 of Intron 7, corresponding to I7G1584T, of the human *GABRB2* gene.

[00112] The various publications and published methods mentioned above are incorporated herein by reference.

Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the spirit and scope of the invention as outlined in the claims appended hereto.